RESOLUTION OF FUSED GAS CHROMATOGRAPHIC PEAKS BY DECONVOLUTION WITH EXTENSION OF THE FOURIER SPECTRUM

By

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After correction for detector response, the area under a peak in a chromatogram is usually taken to be indicative of the amount of that component in the sample. When two or more components are merged under a single peak the problem of determining the fraction of the area to be associated with each component is a difficult one. If overlapping is incomplete so that a valley or local minimum exists, then a variety of techniques such as triangulation, peak height measurement, skimming, or the dropping of a perpendicular from the valley have been used to assign peak areas [1-4]. These methods vary widely in accuracy and none have proved entirely satisfactory. The taking of a chromatogram has also been mathematically modeled as a linear and shift-invariant operation where the instrumental broadening does not change as the input is scanned [5]. In this case the intensity of the observed signal, h(t) can be represented by a Fredholm integral of the first kind, or convolution [6],

$$h(t) = \int_{-\infty}^{\infty} f(t')\, g(t-t')\, dt' = f*g \qquad (1)$$

where f(t') is the unbroadened measurement, g(t-t') a function describing the broadening effect, and f*g a convenient notation for the integral. To compute the integral one multiplies and integrates the product of f(t') and g(t-t') for each increment of t over the range of $\pm\infty$. The function f is regarded as an ideal chromatographic peak that has been broadened and otherwise distorted to h by the measuring process represented by an instrument function g.

If g can be defined, then h can at least partially be restored by solving for f, that is by deconvolving. The degradation of the signal is caused by the gas chromatographic column itself as well as by such non-column factors as dead volume, detector time-constants, and injection profile.

Previous investigators assumed that gas chromatographic peaks are intrinsically Gaussian and are skewed by the effects of dead volume, flow rate, and detector response time. Assuming further that the peak variances of the column contributions (Gaussian) and the non-column contributions (exponential attenuation) add simply, these investigators used the convolution model to determine the effect of dead volume and flow rate on gas chromatograms [5].

The convolution model has also been employed together with the convolution theorem for Fourier transforms to enhance the resolution of steric-exclusion chromatograms and to investigate the effect of noise on this enhancement. The enhancement was found to result in badly oscillating deconvolved peaks unless the data were smoothed prior to deconvolution by the use of a filter, or window, in the transform domain, and unless the instrument function g was chosen so that the resulting peaks were only partly resolved [7].

Full baseline resolution of almost completely fused gas chromatographic peaks should be achieved while avoiding serious oscillations if the Fourier spectrum is appropriately extended after application of an ideal low-pass filter (window). Furthermore, such baseline resolution should produce peak areas that are in good agreement with the areas obtained from chromatographically separated peaks. This technique constitutes the basis for this paper.

THEORETICAL

To the extent that the taking of a chromatogram represents a linear, shift-invariant process, it may be modeled by the convolution equation. Following Bracewell [8] we define the Fourier transform of a function f(t) to be

$$F(s) = \int_{-\infty}^{\infty} f(t) \exp(\mp i2\pi ts)\, dt, \qquad (2)$$

where the negative sign produces the forward transform and the positive sign the backward, or inverse, transform. For simplicity, we use the transform of a continuous function rather than the replicated transform of a discrete function although our data are actually digitized. The ideas expressed here extend to the discrete case provided care is taken to minimize the effects of aliasing and wrap-around [8,9]. Using upper case letters to represent the Fourier transform where F(s) is the transform of the ideal, resolved spectrum, H(s) of the measured chromatogram, and G(s) of the instrument or response function, then according to the convolution theorem for Fourier transforms we have

$$H(s) = F(s)G(s). \qquad (3)$$

In principle one can solve for the transform of the resolved chromatogram by dividing equation (3) by the transform of the instrument function,

$$F(s) = H(s)/G(s), \qquad (4)$$

so that taking the inverse Fourier transform provides the desired ideal chromatogram. It is clear that whenever G(s) is zero F(s) is not defined and the simple procedure breaks down. When this occurs only at a point, or at a series of isolated points, however, continuity of F(s) may be invoked to define F(s) at those values where G(s) is zero.

Unfortunately, real measuring instruments are usually unresponsive to input frequencies above some cut-off frequency S_c. Thus, although such high frequencies may actually be present in the ideal function f(t), they cannot be present in the measured chromatogram h(t) since such frequencies are not passed by the instrument. This situation is illustrated in figure 1 where hypothetical magnitudes of the transforms F, G, and H are shown as a function of frequency. In such cases, G(s) is zero above S_c and the simple procedure indicated above would leave F(s) undefined over this entire region. Bracewell and Roberts [10] have overcome this problem by defining F(s) to be identically zero above S_c and calling such a solution the principal solution $F_p(s)$ so that

$$F_p(s) = H(s)/G(s) \quad \text{for } G(s) \neq 0$$

$$F_p(s) = 0 \qquad \text{for } G(s) = 0. \tag{5}$$

The inevitable presence of noise in the data further complicates the process of deconvolution. When additive noise is present, the measured chromatogram may be more correctly modeled as the sum k(t) of the noise n(t) and the convolution of the ideal spectrum with the instrument function,

$$k(t) = f*g + n(t) = h(t) + n(t). \tag{7}$$

In gas chromatography as with most analytical techniques, the exact form of n(t) is not known and so cannot be removed from the data. Then, the transform of the chromatogram K(s) becomes

$$K(s) = F(s)G(s) + N(s) = H(s) + N(s) \tag{8}$$

and we must choose a new meaning for the principal solution so that

$$F_p(s) = K(s)/G(s) \text{ for } G(s) \neq 0$$

and

$$F_p(s) = 0 \text{ for } G(s) = 0 \qquad (9)$$

The presence of a noise spectrum causes the principal solution to diverge even further from the transform of the desired ideal chromatogram. Particularly disturbing is the fact that for small values of G, H may be less than N so that deconvolution amplifies the noise, a fact previously demonstrated for steric exclusion chromatography [7]. This situation is shown in figure 2 where the divergence of F_p from F(s) is seen to be due to noise as well as truncation. Because the instrument will not respond to input frequencies higher than S_c, the extension of N and consequently K beyond S_c indicates that this noise was acquired somewhere in detecting, amplifying, or recording the data. The interpretation of K as true data for $S > S_c$ is not compatible with the existence of a cutoff frequency , therefore, K(s) is composed entirely of incompatible noise at frequencies above S_c. Noise at frequencies below cutoff is termed compatible noise. Truncation of the principal solution at S_c prevents incompatible noise from influencing the deconvolution. However, compatible noise cannot easily be removed and does cause distortion. Previous investigators have reported that these distortions may take the form of unwanted oscillations in the deconvolution [7]; even in the absence of noise, the truncation of F would produce oscillations.

Clearly, confidence in the results of the deconvolution would be enhanced if one could minimize these oscillations while simultaneously eliminating the high frequency noise. The method of function continuation to cleanly extend the Fourier spectrum into the noise dominated region is accomplished in the following manner [11, 12, 13]. A suitable instrument function, often a modification of an isolated single component peak, is devised and its Fourier transform obtained. The transform of the chromatogram, including the noise, is divided by the transform of the instrument function to obtain the solution in the transform domain. The real, positive portion of the spectrum that results from this procedure is shown in figure 3A, where the large high frequency components in this spectrum are due to noise that has been amplified by the spectrum division. With practice the frequency above which the noise causes severe distortion when the inverse transform is taken can be estimated with fair accuracy. The spectrum is then truncated by setting all frequency components higher than this component equal to zero, and the inverse transform of this principal solution is obtained, thus completing the deconvolution. The result is, of course, the ideal, low-pass filtered deconvolution. The full-width at half maximum and the location of the deconvolved peaks are noted and an artificial function, a(t), is constructed as the sum of Gaussian functions of the same width and location. It is, of course, absolutely smooth, devoid of oscillations and artifically free of noise. However, since the parameters from which this function is formed are determined from deconvolution of the principal solution in which oscillations are present, the size and locations of these peaks are subject to the same errors that are always present in ideal low-pass, filtered results, which have been noted in chromatographic deconvolution by other investigators [7]. The artificial function is therefore used only in a subsidiary manner to continue the Fourier

spectrum and prevent oscillations. When the transform of the artificial function, A(s), is taken, the real, positive portion of the spectrum is as shown in figure 3B. Comparison of figure 3A with 3B shows that the two spectra agree well at low and moderate frequencies, but differ at high frequencies because of the presence of noise in the transform of the actual chromatogram. The frequency at which noise begins to become important is determined, and is denoted with an arrow. A new spectrum is formed by joining the low and medium frequency portions of the deconvolution spectrum to the high frequency portion of the spectrum of the artificial function. This hybrid spectrum is exhibited in figure 3C. The inverse transform of this hybrid spectrum then yields a deconvolution in which the sidelobes have been attenuated. This deconvolution has the virtue of avoiding the high frequency noise present in the chromatogram while simultaneously providing a realistic extension of the Fourier spectrum which attenuates the sidelobes which would arise from a simple Fourier deconvolution with truncation. In order to test this deconvolution procedure, several poorly resolved chromatograms with varying degrees of overlapping peaks were obtained and deconvolved. These results were then quantitatively compared with the fully resolved chromatogram.

EXPERIMENTAL

All of the chromatographic data were obtained from a Varian Model 1440 gas chromatograph equipped with a single flame-ionization detector. The 10 ft x 1/8 in stainless steel chromatographic column was packed with 15% Dexil 300 on a 100/120 mesh Chromosorb W-DMCS support. The chromatograms were of a five component mixture composed of 2-methylpentane, n-hexane, 2,2,4-trimethylpentane, dimethylhexane, and 2,2,5-trimethylhexane (Calibration Mixture 22A, PolyScience Corporation, Niles, Illinois). Sample injection volumes were

0.75, 0.40, and 0.30 μℓ for the three analyses which were temperature programmed from 75°C, 135°C, and 150°C, at 4°, 6°, and 6° per minute, respectively, to yield well resolved chromatographic peaks at the lowest temperature condition and highly fused peaks at the higher temperatures. The helium carrier gas flow was maintained constant at 20 mℓ/min during each temperature programmed run as measured on a Hastings Model LF-50 flow meter (Hastings Raydist, Inc., Hampton, Virginia).

The analog output from the FID was digitized and sampled at a 10 Hz rate. From measurement of the analog signal, the width of the narrowest peak was determined to be on the order of 10 seconds. According to Bracewell's Sampling Theorem [8], this minimum of 100 points is sufficient to assure validity of the recorded data with little or no loss in information. This theorem states that if the function is band limited, that is, the Fourier transform is unequal to zero over a finite range of the transform variable, then there is a sampling interval for which the sample set is fully equivalent to the complete set of function values, so that the information can be obtained with full accuracy. This interval is shown by Bracewell to have a critical value of $0.5/S_c$.

The various factors affecting broadening and distortions of gas chromatographic peaks are now reasonably well known from kinetic and thermodynamic considerations of mobile and stationary phase behavior, diffusion effects, and flow irregularities [14]. When the sorption isotherm is linear the separated solute band initially approximates a smooth Gaussian distribution curve. As the solute zones proceed through the column they are broadened and the concentration at peak maximum gradually decreases. When the sorption isotherm is nonlinear, the broadened peaks are also asymmetric.

Fourier transforms have been used by others to deconvolve gas chromatographic elution curves [7, 17]. Deconvolution of the gas chromatogram requires that an instrument function be defined which reflects the true form of the chromatographic peak. Intuitively, one would assume that any isolated peak could be used to define the instrument function. In some circumstances this assumption can give reasonable results, but in general, use of the unmodified isolated peak is not totally satisfactory since it in itself is broadened by the instrument function.

Since our purpose in the present work was to obtain a more accurate deconvolution for resolution and quantification enhancement, we required a simple procedure for generating an instrument function yielding resolution-enhanced symmetrical peaks on deconvolving. Attempting the deconvolution with a Gaussian function resulted in Gibbs oscillations as well as shoulders suggesting additional peaks in the deconvolved data. Only a small improvement was realized by using an unaltered isolated peak to construct the response function. Hence, an empirical procedure for producing an instrument response function in the form of a skewed Gaussian [15] was devised which yielded symmetrical peaks on deconvolving [16]. This function was derived by determining the retention time of an isolated peak and then deliberately skewing the peak by expanding above this point and compressing below by a common factor. The width of the skewed peak was made variable to control the resolution, and a shifting factor was used to determine the position on the time axis, and hence the location in the deconvolved chromatogram. Various combinations of these three parameters were tried until the deconvolution was optimized.

Even with a well defined response function, however, sidelobes in the principal solution persisted due to the abrupt truncation of the Fourier

transform F_p. An artificial function was devised using Gaussian peaks of equal height, location, and width at half-maximum as the optimized deconvolved function, in such a manner that its coefficients matched those of the deconvolved function, especially in the region near the cutoff frequency. Coefficients from the artificial function were then substituted for those of the original transform F beyond the cutoff (figure 3). This transform thus became the hybrid with the original coefficients used up to cutoff and artificial function coefficients beyond. Coefficients corresponding to frequencies greater than the hundredth were set equal to zero since they originated in noise and had a negligible contribution to the new hybrid function. The enhanced chromatogram was then obtained from the inverse transform of the hybrid function, and the areas under the peaks calculated by numerical integration.

RESULTS AND DISCUSSION

The gas chromatograms which were evaluated for the comparison are given in figures 4 and 5. Figure 4 is the chromatogram resulting from the injection of 0.75 µℓ of the five component alkane mixture at an injection temperatue of 75°C and a linear temperature program of 4°C/minute. As is readily seen, separation efficiency is sufficient that the peaks are well resolved. Only the 2-methylpentane and hexane peaks are close together and these are essentially base-line resolved with a calculated resolution value of 1.3. This well-resolved chromatogram was used as the reference standard for evaluation of the effectiveness of our fast Fourier transform (FFT) deconvolution technique. Results of deconvolving the data of figures 5A and 5B are shown in figures 6 and 7, respectively.

The chromatograms shown in figure 5A and 5B resulted from the injection of 0.40 and 0.30 µℓ of the same mixture at 135°C and 150°C, respectively, and

a linear temperature program of 6°C/minute. In comparison with figure 4, the separation efficiency in figure 5A has decreased considerably with peaks 1 and 2 fused but easily distinguishable as two overlapping peaks. Figure 5B depicts the almost complete fusion of peaks 1 and 2 with their overlap so severe as to be distinguishable as more than one peak by virtue of the peak dissymmetry or prior compositional knowledge. While accurate peak area measurements for quantitative analysis are readily obtained for the chromatographic data in figure 4, accurate integration and evaluation of the peak area for each of the individual peaks in the overlap regimes of figure 5A and 5B is highly unlikely if not impossible without the use of some deconvolution technique.

The instrument function g(s) used in this work was derived by assuming that a Gaussian function defined an isolated and well-resolved peak (peak 5 in the chromatogram in figures 4 and 5) and then deliberately skewing the peak in the manner described earlier, until suitable resolution of the chromatogram was obtained upon deconvolution. The resolution-enhanced chromatogram or low pass inverse filtering deconvolution, of the first two peaks of the mixture is given in figure 6D. This chromatogram resulted when the transform of the data in chromatogram 6A was divided by that of the instrument function, followed by inverse transformation of the solution back into the time domain to give the resolved and symmetrical peaks. The deconvolved peaks were quite well resolved although there were remaining sidelobes resulting from truncation of the higher frequencies, as had been observed by Maldacker et. al. [7] in their application to steric-exclusion chromatography. However, resolution was improved with the R value increasing from 0.64 for figure 6A to 1.2 for figure 6D. Similar results were obtained for the 150°C case. Resolution enhancement values for our data are listed in Table 1.

Table 1 Resolution Enhancement Data

Run[a]	Injection T	No. Plates, N[b]	Resolution[c], R	
1	75°C	1120	1.3[d]	-
2	135°C	820	0.64[d]	1.2[e]
3	150°C	740	0.40[d]	1.0[e]

a) Runs 1, 2, and 3 are depicted in figures 4, 5A, and 5B, respectively.

b) Calculated from chromatographic peak #5 in each run.

c) Resolution was calculated using $R = 2\,(T_2 - T_1)/(W_1 + W_2)$ where T_1 and T_2 are the retention times of the first two components eluted and W_1 and W_2 their extrapolated base-line widths in time units.

d) Resolution values for components 1 and 2 under three temperature conditions and without computer enhancement.

e) Resolution values for components 1 and 2 after computer enhancement.

We realized that even with our good instrument function, truncation of the principal solution at the higher frequencies caused errors due to sidelobe formation which could lead to peak integration problems for quantitation. In order to minimize the sidelobes, the artificial function for continuing the spectrum above a selected cutoff frequency was generated. Since a Gaussian closely approximated the peak shape, this new function was derived by assuming Gaussian peaks and using the peak locations and widths at half-height in the deconvolved chromatograms. The resulting artificial function is the hypothetical chromatogram shown in figure 6B (or 7B). Its transform was then obtained by conventional FFT techniques and coefficients corresponding to frequencies from the cutoff frequency to the 100th frequency were taken from the transform of the artificial function and grafted to the truncated

transform of the chromatogram. The resolution-enhanced chromatograms which resulted from deconvolution with function continuation are shown in figures 6C and 7C. It is clear that the sidelobe amplitudes have been decreased by a factor of two through the use of the deconvolution with function continuation technique when compared to the deconvolution with ideal low-pass inverse filtering technique shown in 6D. That this has occurred without sacrifice of either resolution enhancement or peak area quantitation is also demonstrated in Tables 1 and 2. While the resolution enhancement cannot be such as to separate the peaks as much as they appear in figure 4, the enhancement did allow essentially base-line resolution for area integration purposes. Table 2 demonstrates not only the increased accuracy of the area measurements but also the capability for accurate quantitation in the case of seriously overlapped peaks in figure 5B where the resolution is only 0.40 when compared with the data from figure 4 where the resolution for the first two peaks was essentially base-line at R equals 1.3.

Table 2 Normalized Areas Under the Chromatographic Peaks[a]

Species	Run 1[b,c]	Run, 2[c]	Run 2[d]	Run 3[c]	Run 3[d]
Methylpentane	12.8	9.8	13.4	33.8[e]	13.4
n-Hexane	21.4	24.2	21.0		20.8
Isooctane	33.5	32.1	33.1	32.0	33.1
Dimethylhexane	14.7	15.4	14.7	15.3	14.5
Trimethylhexane	17.7	18.5	17.9	18.9	18.1

a) Areas obtained from 10 Hz digitized data using trapezoidal rule for peak integration.

b) Temperature programmed for essentially base line resolution (figure 4).

c) Integrated areas without resolution enhancement.

d) Integrated areas after resolution enhancement via FFT with function continuation.

e) Area under almost completely fused peak.

SUMMARY

Earlier investigators used either synthetic functions or an experimental non-retained peak in order to enhance resolution of overlapping chromatographic peaks. In this case, we generated an instrument function by intentionally skewing an isolated and fully-resolved gas chromatographic peak, which was assumed to be Gaussian, until the resulting deconvolution of its Fourier transform with the transform of the original data yielded a resolution-enhanced chromatogram with symmetrical peaks. This resulting chromatogram was then used to generate an artificial Gaussian chromatogram whose transform of the higher frequencies were grafted onto the transform of the original data from the truncation frequency to the 100th frequency.

Addition of these artificial function frequencies to the transform of the original data when deconvolved with the instrument function yielded well-resolved chromatograms with minimal peak sidelobes. The resolution of two peaks which were almost completely fused with R = 0.40 in the original data was raised to 1.3 in the resolution-enhanced chromatogram obtained from deconvolution with function continuation. The additional improvement in resolution was also reflected in the realization of increased accuracy in the integration of the actual areas under the chromatographic peaks.

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FIGURES

Figure 1

Convolution Operation, Transform Domain

Figure 2

Effects of Noise on the Principal Solution

Figure 3

Real Positive Portion of the Transform of: (A) Ideal Chromatogram, F(s); (B) Artificial Function, A(s); (C) Hybrid Function Formed by Combining Parts of F(s) and A(s) at S_c.

Figure 4

Instrument Resolved Chromatogram of 5-Component Alkane Mixture, 75°C Injection Temperature.

Figure 5

Poorly Resolved Chromatograms of 5-Component Alkane Mixtures, Injection Temperatures: (A) 135°C; (B) 150°C

Figure 6

Deconvolution of First Two Fused Peaks, 135°C Case: (A) Original Data; (B) Artificial Function; (C) Deconvolution with Function Continuation; (D) Low Pass Inverse Filtering

Figure 7

Deconvolution of Fused Peaks, 150°C Case: (A) Original Data; (B) Artificial Function, (C) Deconvolution with Function Continuation

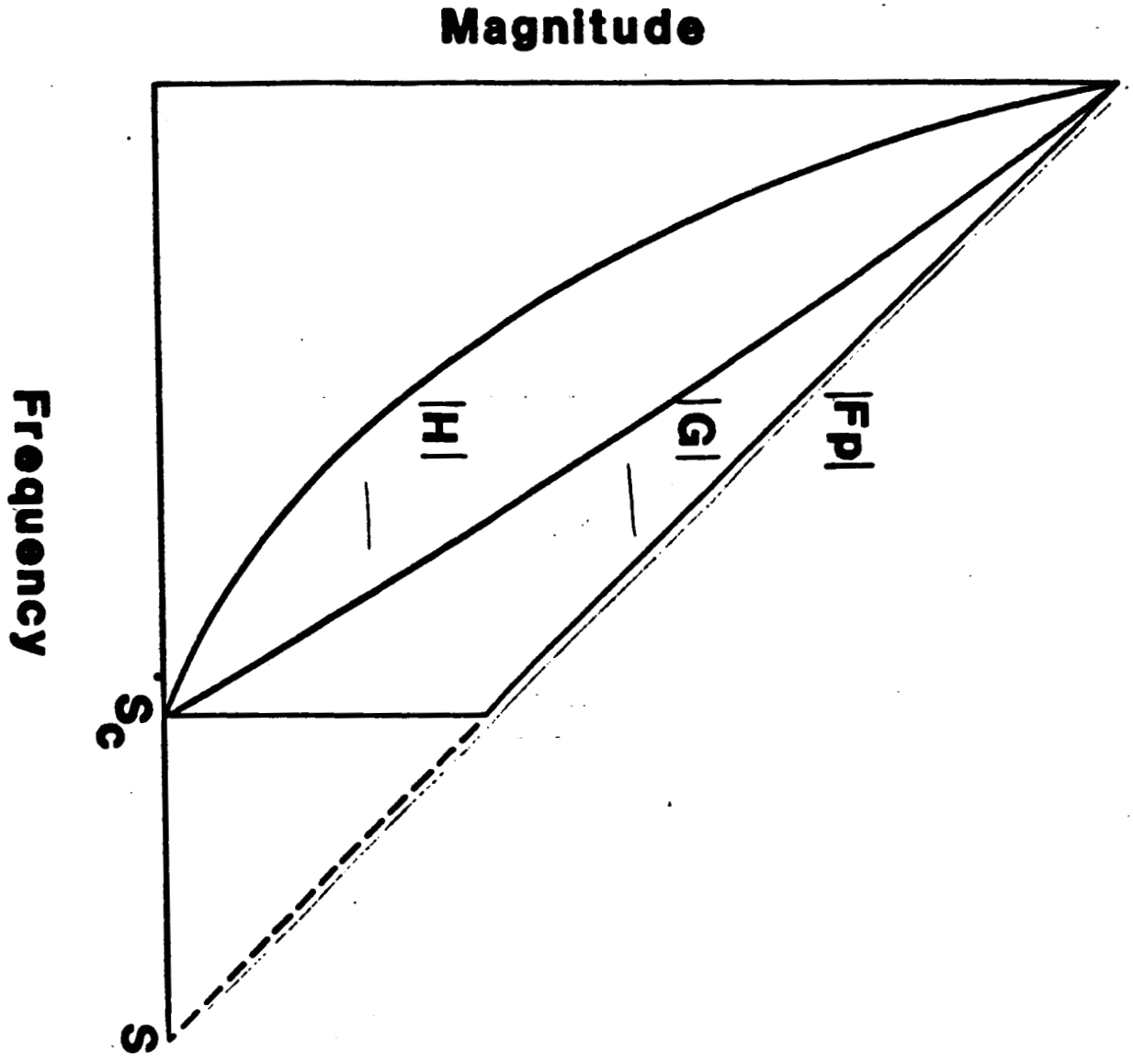


FIG 1

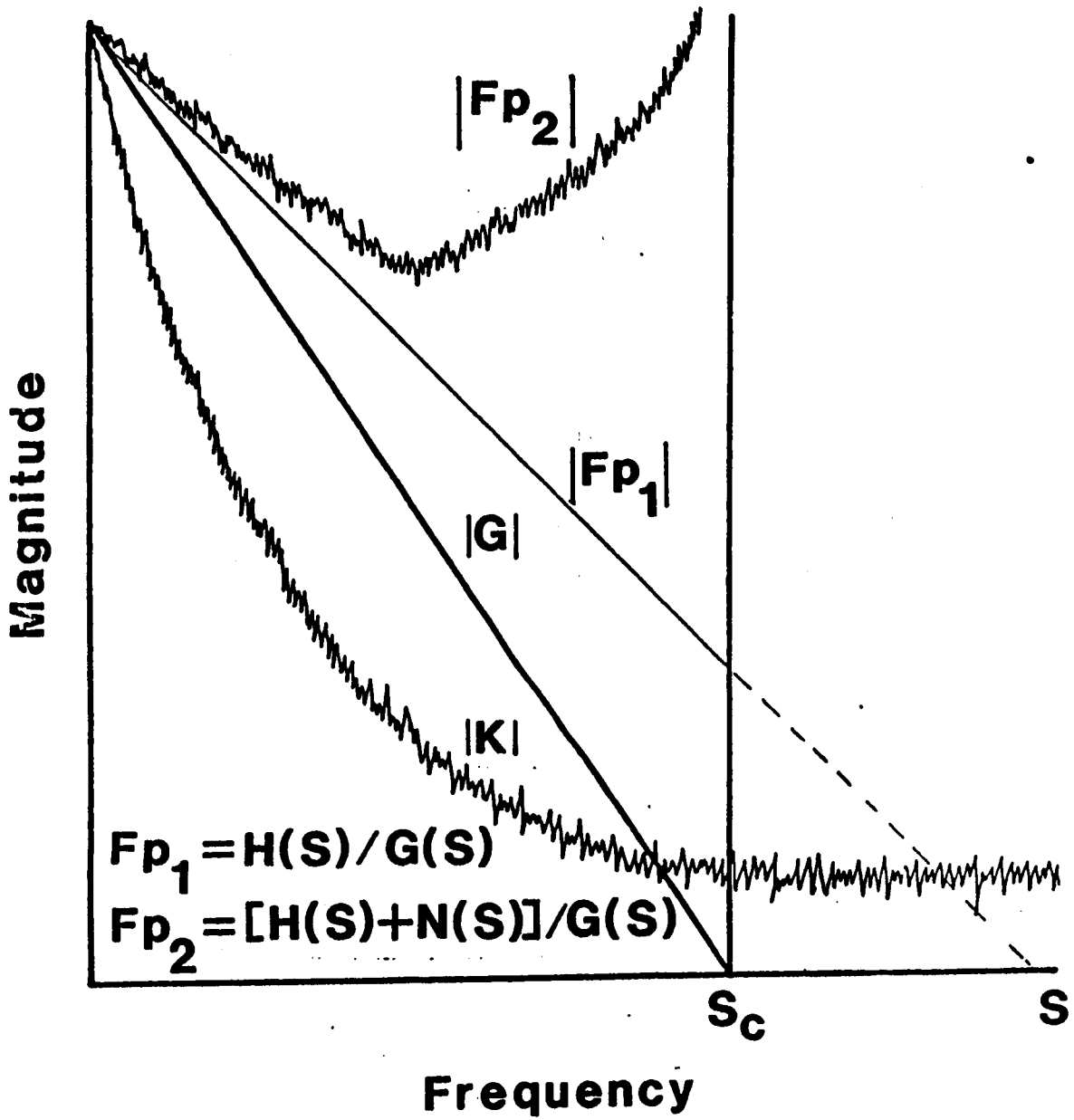
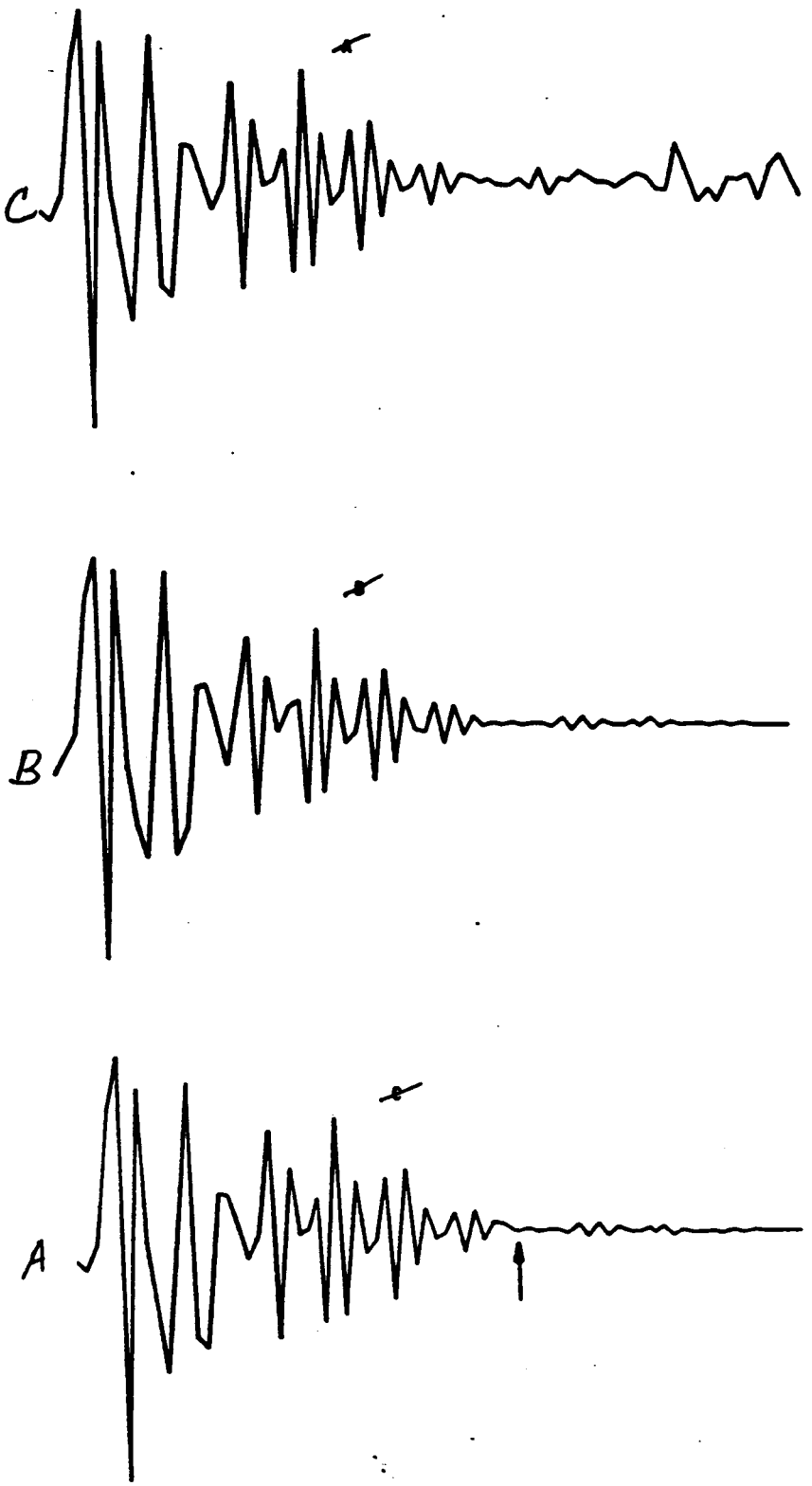


FIG 2



FREQUENCY IN ARBITRARY UNITS

FIG 3

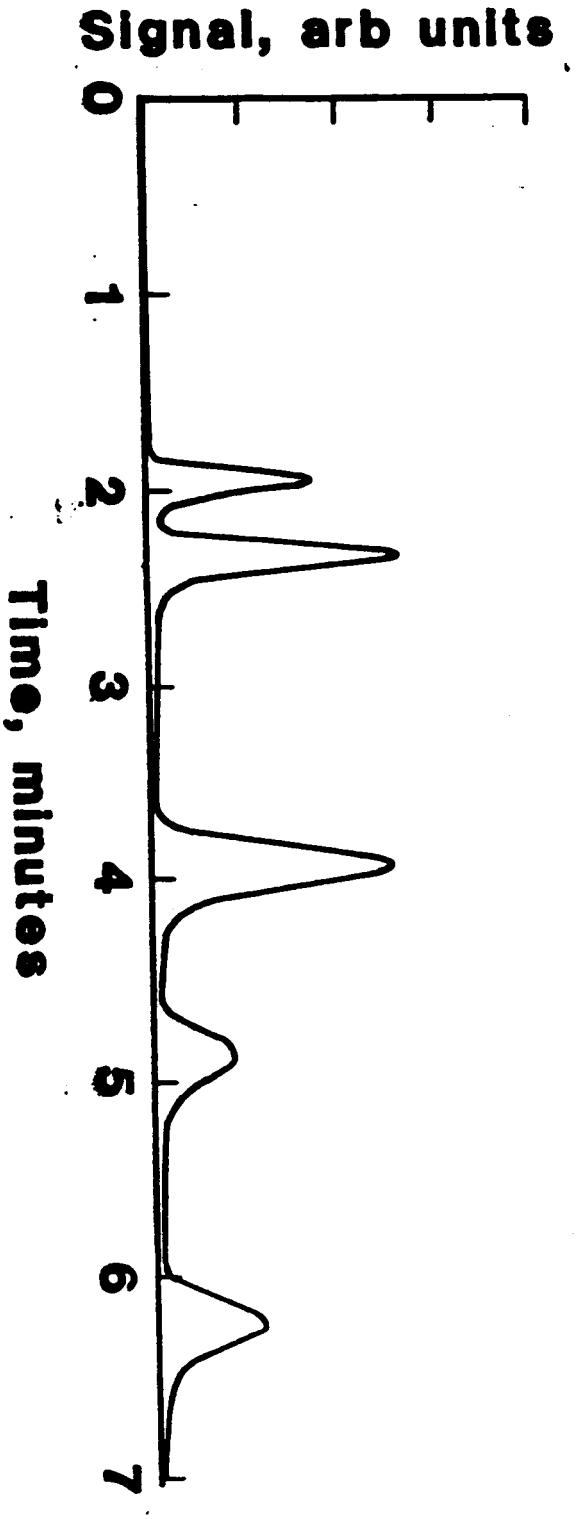
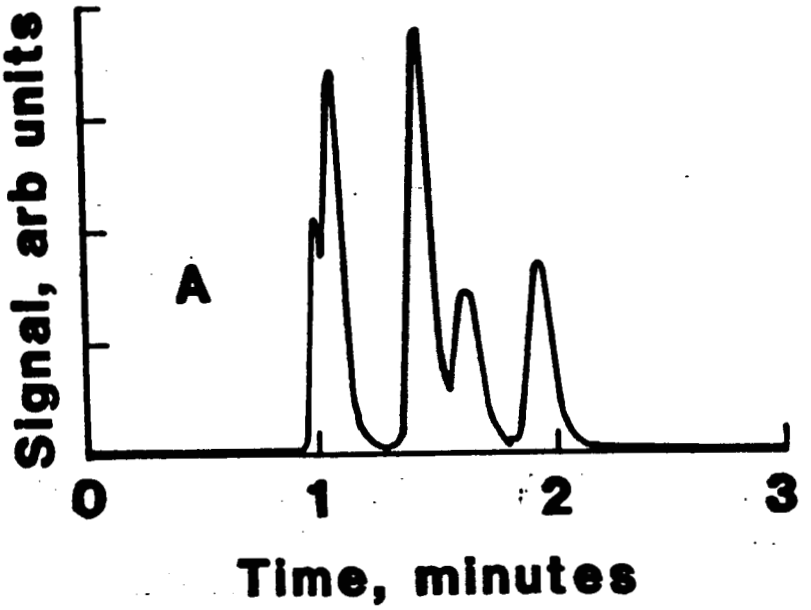


FIG 4


Signal, arb units
A
0
1
2
3
Time, minutes

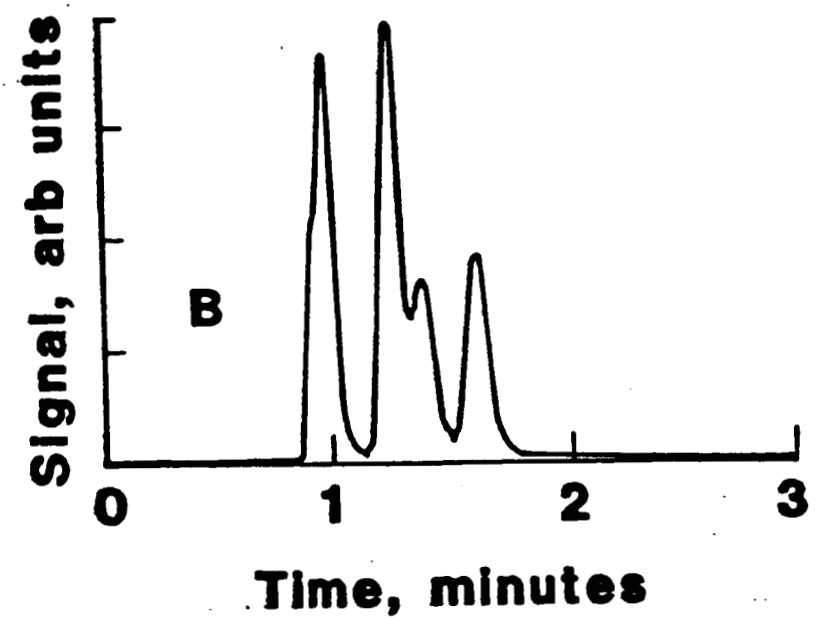

Signal, arb units
B
0
1
2
3
Time, minutes

FIG 5

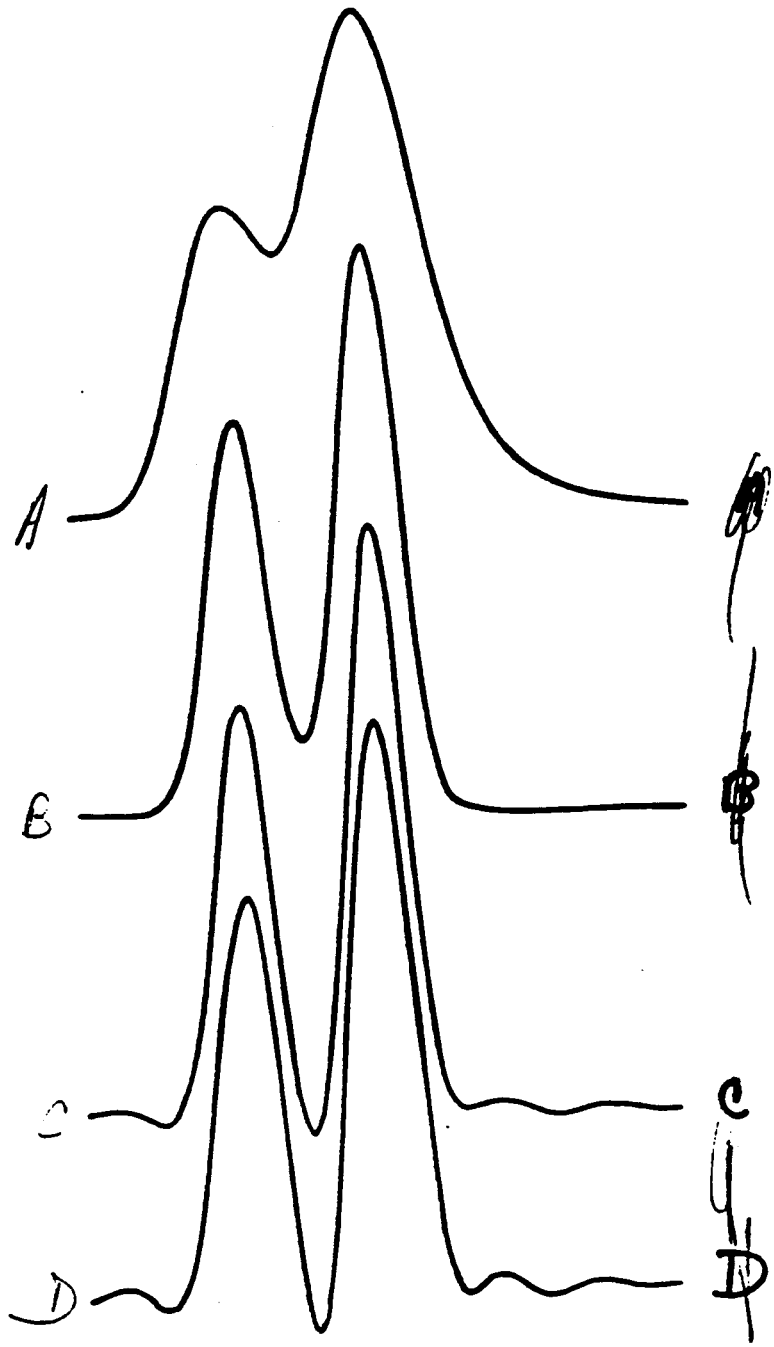


FIG. 6

FIG 7

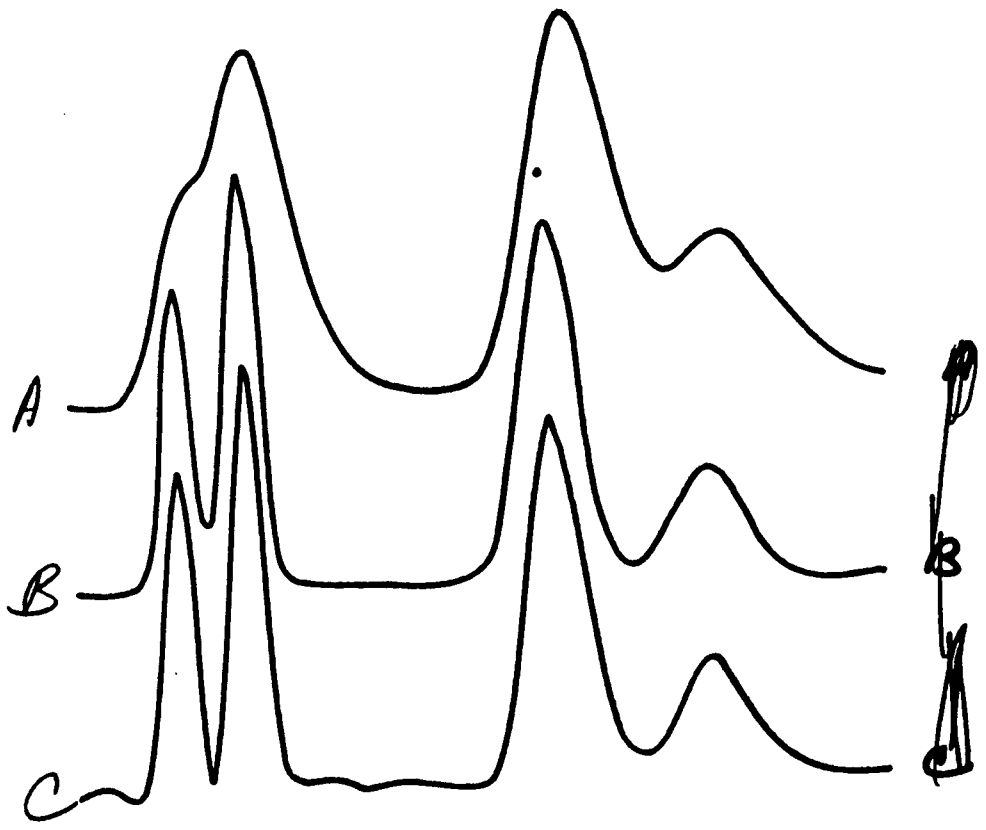


FIG 7

Fig 8